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Full Length Research

Antimicrobial, Phytochemical and Crude Lipid Content of Sunflower (*Helianthus annuus*) and Coconut (*Cocos nucifera*) Oils

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Oil are heterogeneous collection of biochemical substances which have in common the property of being soluble in most organic polar solvents and insoluble in water. They are used for different purposes from food, medicines and biofuels. This study was aimed at determining the antimicrobial as well as chemical constituents of the two oils. The antimicrobial activities of the oils were assayed using Agar-well diffusion technique against *Salmonella typhi*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Qualitative phytochemical screening was carried out and crude lipid contents were determined. The result of the antimicrobial screening showed antimicrobial potency of sunflower oil against the organisms at highest concentration of 3.12% v/v to *S. aureus* with zone of inhibition of 26mm, *K. pneumonia* with zone of inhibition of 22mm, *S. typhi* with zone of inhibition of 20mm, *P. aeruginosa* with zone of inhibition of 18mm. The Minimum Inhibitory Concentrations (MIC) of the extract ranged 1.56%-3.12% and the Minimum Bactericidal Concentrations (MBC) ranged of between 3.12%-6.25%. Coconut oil showed activity against only *K. pneumonia and S. aureus* with zone of inhibition of 19mm and 24mm respectively at the highest concentration of 12.5% both. The MIC for the two organisms were at 12.5% while the MBC for the *K. pneumonia* was 25% and 12.5% for *S. aureus*. The sunflower oil contains terpenes, sterols and flavonoids while the coconut contains carbohydrate in addition. The lipid content of sunflower was 70.6% while coconut oil has 68.8%. The result of this study showed that both oil has proved its use in folklore as an alternative antimicrobial agent and further research can lead to isolation of a new lead of medical importance.

Keywords: Oil, Antimicrobial, sunflower, coconut, MIC.

INTRODUCTION

Oil are heterogeneous collection of biochemical substances which have in common the property of being soluble in most organic polar solvents and insoluble in water (Aboki et al., 2012). The beneficial uses of oil from plants have been known since time immemorial either as food items, biofuels or medicines (Talukdar et al., 2015). Edible oils extracted from plant sources are important in foods and in various other industries. The sunflower seed is the fruit of *Helianthus annuus* L, belonging to the family Asteraceae (Islam et al., 2016). This plant is primarily cultivated for its seeds which yield the second most important source of edible oil rich source of vitamins, especially vitamin E or as condiment (Taha et al., 2012; Talukdar et al., 2015). The other parts of the plant, notably the petioles and young flowers, were used as savory delicacies before the use of seed as food (Dwivedi and Sharma, 2014; Islam et al., 2016). The leaves of *H. annuus* are extensively used in South Eastern Nigeria in the traditional treatment of diarrhea, diabetes mellitus, inflammation, bacterial infection and respiratory problems (Eze et al., 2015). The seed oil, shoots, and herb tincture have been employed for anti-inflammatory, antipyretic, astringent, cathartic, diuretic, emollient, expectorant, stimulant, vermifuge, cancer, throat and lung infections. The yellow petals are used as coloring agents which gives it new prospect in cosmetic industry (Aziz et al., 2013; Dwivedi and Sharma 2014). There are experimental reports on the antimicrobial activity, the antioxidant, antidiabetic and hepatoprotective properties of the sunflower stem and seed oil (Eze et al., 2015).

Coconut (*Cocos nucifera* L), family Palmae is a pantropical plant whose centre of origin is uncertain (Tock, 1997). It is grown in more than 80 countries and a life-sustaining species in fragile coastal and island ecosystems (Arunachalam, 2012). The plantations are usually located in the lowlands just above beach level. The trees are tall, reaching up to 30 m in height, with a slender, often curved trunk. Fruit-bearing starts after six years. Coconut has many uses, including providing food and oil for millions besides ornamental and aesthetic uses. Its uses are so numerous and, as every part of the coconut palm is of some use, the coconut palm has been described as ‘one of Nature's greatest gifts to man’ (Jerard et al., 2008). Tropical communities have used coconut oil in key areas of their lives, such as cooking, for medicinal purposes, and for skin and hair conditioner (Francis, 2011). Studies have indicated that the lauric acid in coconut oil has antibacterial action and that the medium-chain fats in coconut oil are similar to fats in human milk and have similar nutritional effects. Coconut Promotes the flow of urine, is used in the disease of the uterus. It is also given to persons with liver complaints, bronchitis and dysentery. The infusion of the young roots is used as gargles for sore throat. The freshly tapped juice of the flower stalks is recommended for constipation (Francis, 2011). The benefits of coconut oil for health are countless and unparalleled.

To minimize overuse of antibiotics in the treatment of infectious diseases and control of drug resistant bacterial strains, has necessitated the exploration of natural and safe antimicrobial substances as new alternatives to replace synthetic chemicals from seed oil producing plants and their oils that are known to possess antimicrobial properties (Kamila et al., 2004; Prabhakaran, 2010; Priyanka et al., 2015). This is largely because antimicrobial resistance against drugs results in greater severity and increases hospitalization periods, morbidity ratios and costs to society. The medicinal power of these plants lies in phytochemical constituents that are synthesized during secondary metabolism of the plants (Akinmoladun et al., 2007; Adetunji et al., 2014)

The present study was aimed at determining phytochemical, lipid content and antimicrobial properties of sunflower and coconut oils against pathogenic microbes in search of new, safe and readily available alternative antimicrobial agents from natural resources.

MATERIALS AND METHODS

Materials

Sunflower and Coconut Oils

The sunflower and Coconut oils were purchased from a local producer from Minna Niger State, Nigeria.

Test Organisms Used for the Test

The microorganisms used were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,*

Staphylococcus epidermidis, Proteus vulgaris and *Candida albicans*. They are clinical isolates obtained from the Department of Microbiology and Biotechnology of National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. The bacteria were maintained on Nutrient agar slant at 37^{O}C and *Candida albicans* was maintained on potato dextrose agar slant at 30^{O}C.

Methods

Phytochemical Screening

The oils were analysed for the presence of alkaloids, carbohydrates, glycosides, steroids, terpenoids, phennols, tannins and flavonoids. The phytochemical screening was carried out according to the methods outlined in (Trease and Evans, 1989; Sofowora, 1993; Evans, 2004).

Determination of Crude Lipid Content

The recommended method of the Association of Official Analytical Chemists (AOAC, 2006) was used for the determination of crude content. Crude lipid was assayed by exhaustively extracting one gram of each sample for 3 hours with petroleum ether in a soxhlet apparatus.

In vitro Determination of Antimicrobial Activity

Inoculum preparation

A loop full of isolated colonies was inoculated into 4 ml sterile Mueller-Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi and incubated at 37^{O}C for 2h. The turbidity of actively growing microbial suspension was adjusted with freshly prepared MHB and SDB using $BaSO_4$ turbidity standard to match turbidity standard of 0.5 McFarland. This turbidity was equivalent to approximately $1.5x10^8$ CFU/mL cells for bacteria, and $1.5x10^7$ spores/ml for fungal strain. The grown suspension was used for further testing.

In vitro Antimicrobial Susceptibility Assay of the Crude Extract

Susceptibility testing of the extract against the isolates was determined in the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, using Kirby-Bauer agar diffusion method according to NCCLS standards (Bauer et al., 1966; NCCLS, 2000). The Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) were used for the antimicrobial activity test. About 100 µL of MHB and SDB cultures containing 0.5 McFarland equivalent approximately $1.5x10^8$ CFU/mL cells for bacteria, and $1.5x10^7$ spores/ml for fungi strain were dispensed into empty sterile petridish using micropipettes. Twenty three millilitres (23ml) of sterilized MHA and SDA maintained between 50 – 45^{O}C was added to the appropriate petri dishes and rocked gently for even distribution of the organisms under aseptic condition and allowed to gel under safety hood for one hour. On each of the plates containing bacteria isolates. Five wells of 8 mm in diameter were made on the agar plates using sterile metallic corkborer and labelled properly. The base of the wells was sealed with 30 µL of MHA and SDA. Thereafter, 200µl of different concentrations of the oils were carefully dispensed with the aid of micropipette into each well and left in the safety hood for 2 h for proper diffusion of the oil into the agar and then incubated at 37°C for 24h for bacteria. The same procedure was repeated for fungi strain and incubated at 25°C for 48 h for fungi. The experiment was set up in duplicates. The plates were observed for activity and zones of inhibitions were measured and recorded as mean of zone of inhibition. The diameters of each zone were accurately measured with a spotless and translucent ruler in millimetre (mm).

Control experiments were set up using standard antibiotics, Ciprofloxacin (250mg) (Fidson, Nigeria) and Fluconazole (80mg) (Pfizer, UK for fungi specie as reference standards for positive control. Media sterility controls and organism viability control were all set and incubated under the same conditions as the sample.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the oils was determined for those organisms that were sensitive to them. The broth microdilution method (BMM) in 96 micro wells titre plates described by National Committee for Clinical Laboratory Standards (NCCLS, 2000) with little modification was used. A volume of 50µl of the extracts was dispensed into first row and the same volume of the sterilized media (MHB and SDB) was

Table 1. Phytochemical screening of Sunflower Oil and Coconut Oil.

Phytochemicals	Test	Sunflower Oil	Coconut Oil
Carbohydrate	Molish test	-	+
Cardiac glycoside	Keller- killani test	-	-
Terpenes and sterols	Lieberman Burchard	+	++
	Salkowski's test	+	++
Tannins and phenols	Ferric chloride test	-	-
Flavonoids	Lead acetate test	+	-

Keys: Present = +, Absent = -

dispensed into each well except the first row. A two-fold dilution was carried out from row 2 by taking 50µl of the extract to the next row, mixed well and the serial dilution continued to row 7 where 50µl from the wells was discarded away. Then, 50µl of 0.5 McFarland of 2h culture was added to each well in row 1-7. The rows 8 and 9 were the OVC and MSC. The microwell plates were closed and incubated aerobically at 37°C for 24 h. Thereafter, 50µl of tetrazolium dye was added into each well and further incubated for 2h at 37ºC and colour change was observed. Any well with reddish-pink colour signifies the microbial growth. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the test.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined by sub-culturing the tubes from the MIC that do not show evidence of growth. The mixture was streaked on MHA and SDA plate in duplicate and incubated for 24 hours at 37°C and 48 hours at 44°C respectively (Banso and Adeyemo, 2006). The plates with minimum concentration of the extract that do not allow the growth of the organisms were considered as Minimum Bactericidal Concentration of the extract.

RESULTS

Phytochemical analysis of the extracts of Sunflower (*Helianthus annuus)* and coconut (*Cocos nucifera*) oils

The phytochemical contents of the samples are presented in Table 1. The results of the phytochemicals confirmed the presence of steroid, terpenoids and flavonoids in sunflower oil while steroids, terpenoids and carbohydrate are present only in coconut oil. However, glycoside, tannins, phenols and flavonoids were not detected in the two samples.

Crude Lipid Content of *Helianthus annuus and Cocos nucifera oils*

From the results obtained, the crude lipid content of sunflower oil (70.6%) is higher than coconut oil (68.85%). The values indicate high oil content and it is comparable with other oil seeds such as cotton seeds and groundnut.

Antimicrobial Activity of *Helianthus annuus and Cocos nucifera oils.*

The crude extract of both *H. annuus* and *C. nucifera* oils showed varied activities against microorganisms. The activity of the oils on the organisms is represented in Tables 2 and 3. The organisms tested are sensitive to *H. annuus* at different concentrations except *S. pyogenes* and *Candida albicans*. However, *C. nucifera* oil only showed activity against *S. aureus* and *K. pneumonia*. The results showed that *S. aureus* is the most susceptible organisms to the two oils, while S. *pyogenes and C. albicans* were resistant to the two oils. The activity of standard antimicrobial agents used as controls in these experiments which are ciprofloxacin and fluconazole are represented in Table 4. Ciprofloxacin had activity against all tested organism and also *Candida albicans* was susceptible to fluconazole,

The MIC and MBC of the two oils are represented in Table 5 with varied values of MIC and MBC for different organisms. The MIC and MBC values

Table 2. Antimicrobial activity of extracts of sunflower oil (Zone of Inhibition diameter (mm)).

Microorganisms	Diameter of zone of inhibition (mm)				
	25%	12.5%	6.25%	3.125%	1.5625%
Staphylococcus aureus	-	-	7	26	8
Pseudomonas aeruginosa	-	-	4	18	11
Streptococcus pyogenes	-	-			
Klebsiella pneumonia	-	-	8	22	14
Salmonella typhi	-	-	6	20	16
Candida albicans	-	-	-	-	-

Key: - = No inhibition

Table 3. Antimicrobial activity of extracts of Coconut oil (Zone of Inhibition diameter (mm)).

Microorganisms	Diameter of zone of inhibition (mm)				
	12%	12.5%	6.25%	3.125%	1.5625%
Staphylococcus aureus	-	24	16	12	3
Pseudomonas aeruginosa	-	-	-	-	-
Streptococcus pyogenes	-	-	-	-	-
Klebsiella pneumonia	11	19	14	6	-
Salmonella typhi	-	-	-	-	-
Candida albicans	-	-	-	-	-

Key: - =No inhibition

obtained for the oils on *S. aureus*, *P. aeruginosa, K. pneumonia* and *S. typhi* varied. For instance, the MIC and MBC values of 1.56% and 3.12% were obtained for *H. annuus* on *S. aureus* and 1.56% and 3.12% were obtained for *S. typhi* respectively.

DISCUSSION

The phytochemical analysis, lipid content and antimicrobial activities of sunflower (*H. annuus*) and Coconut (*C. nucifera*) oils were investigated using standard methods (AOAC, 2006)). The study revealed that the phytochemical constituents of sunflower oils include terpenes, sterols and flavonoids while coconut oil contains carbohydrate, terpenes and sterols. The antibacterial activities found especially in sunflower oil may be mediated by some of the phytochemical constituents of the extracts. The phytoconstituents particularly tannins and flavonoids are known to have antibacterial activity due to their possession of ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins (Eze et al., 2015).

The lipid content of the two oils were comparable to those of cotton seeds and groundnut which have made them healthy for consumption. The oils have high monosaturated fatty acids and hence high nutritional value.

Antimicrobial analysis of sunflower and coconut oils showed varied levels of antimicrobial properties comparable to the standard antibacterial agent used as control. The sunflower oil showed a higher activity than the control drug. However, it was noticed that at higher concentrations, sunflower oil does not showed activity but when in oil/surfactant mixture it does. In this experiment it was discovered that on dissolution in Tween20 and further dilution to lower concentration, allow the oil to diffuse through the agar to allow contact with the growing organisms. The results therefore agreed with the findings of Anjali et al., (2010) indicating that the

Table 4. Antimicrobial activities of Ciprofloxacin and Fluconazole controls (Zone of Inhibition diameter (mm)).

Organisms	Diameter of zone of inhibition (mm)	
	Ciprofloxacin	Fluconazole
Staphylococcus aureus	18	-
Pseudomonas aeruginosa	6	-
Streptococcus pyogenes	8	-
Klebsiella pneumonia	5	-
Salmonella typhi	15	-
Candida albicans	-	13

Key: - =No inhibition

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of Sunflower Oil.

	MIC and MBC of Sunflower oil (%)			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Salmonella typhi*
MIC	1.5625	3.125	3.125	1.562
MBC	3.125	6.25	6.25	3.125

Table 6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of Coconut Oil.

	MIC and MBC of Coconut oil (%)	
	Staphylococcus aureus	*Klebsiella pneumonia*
MIC	12.5	12.5
MBC	12.5	25

microemulsions are stable, self-preserving antibacterial agents, with a highly effective killing rate against bacterial growth when water phase volume is increased. Therefore, decreased diffusability may not demonstrate the antimicrobial efficacy of the oils.

Coconut oils only had activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* also at lower concentrations.

In this study, the MIC values ranged mainly between 1.56% and 3.12%, MBC values range of 6.25% and 3.12% for sunflower oil, MIC values of 12.5% and MBC range of 12.5% -25% in coconut oil is an indication that there is a possibility of sourcing alternative antimicrobial substances from these oils for the development of newer antibacterial agents. The observed low MIC and MBC values against these bacteria means that the plant has the potential to effectively treat any ailments associated with these bacterial pathogens.

CONCLUSION

The potency of sunflower oil is particularly interesting as the crude oil has showed activity against pathogenic microorganisms at very low concentration which have corroborated the use in folklore. There is need for isolation and characterization of lead bioactive compound from

the oil which may serve as an alternative antimicrobial agent.

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